4 (cancelled)

## **Amendments to the Claims:**

This listing of claims will replace all prior versions, and listings, of claims in the application:

## **Listing of Claims:**

1	1 (currently amended): A nucleic acid-lipid particle composition for introducing
2	a nucleic acid into a cell, said particle composition comprising:
3	(a) a nucleic acid-lipid particle comprising a cationic lipid, a conjugated lipid that
4	inhibits aggregation of particles, and a nucleic acid, wherein said nucleic acid is encapsulated in
5	the a lipid bilayer of said nucleic acid-lipid particle, and wherein said conjugated lipid that
6	inhibits aggregation of particles is a member selected from the group consisting of a PEG-lipid,
7	an ATTA-lipid and a cationic-polymer-lipid conjugate having the formula
	AY
8	I
9	wherein:
10	A is a lipid moiety;
11	W is a hydrophilic polymer; and
12	Y is a polycationic moiety; and
13	(b) an endosomal membrane destabilizer, wherein said endosomal membrane
14	destabilizer is Ca <sup>++</sup> ion.
1	2 (original): The nucleic acid-lipid particle composition of claim 1, wherein said
2	endosomal membrane destabilizer is outside said nucleic acid-lipid particle.
1	3 (original) The nucleic acid-lipid particle composition of claim 1, wherein said
2	endosomal membrane destabilizer is both outside and inside said nucleic acid-lipid particle.

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1	5 (original): The nucleic acid-lipid particle composition of claim 4, wherein the
2	concentration of Ca <sup>++</sup> ion is from about 0.1 mM to about 100 mM.
1	6 (original): The nucleic acid-lipid particle composition of claim 5, wherein the
2	concentration of Ca <sup>++</sup> ion is from about 1 mM to about 20 mM.
1	7 (original): The nucleic acid-lipid particle composition of claim 1, wherein said
2	particle has a median diameter of less than about 150 nm.
1	9 (original). The puelois said linid partials composition of claim 1, wherein said
	8 (original): The nucleic acid-lipid particle composition of claim 1, wherein said
2	cationic lipid is a member selected from the group consisting of N,N-dioleyl-N,N-
3	dimethylammonium chloride (DODAC), N,N-distearyl-N,N-dimethylammonium bromide
4	(DDAB), N-(1-(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTAP), N-(1-
5	(2,3-dioleyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTMA), and N,N-dimethyl-2,3-
5	dioleyloxy)propylamine (DODMA), and combinations thereof.
1	9 (original): The nucleic acid-lipid particle composition of claim 1, wherein said
2	particle further comprises an additional noncationic lipid.
1 .	10 (original): The nucleic acid-lipid particle composition of claim 9, wherein
2	said noncationic lipid is selected from the group consisting of DOPE, POPC, and EPC.
1	11 (original): The nucleic acid-lipid particle composition of claim 1, wherein
2	said particle comprises a functional group that facilitates Ca <sup>++</sup> ion chelation.
1	12 (original): The nucleic acid-lipid particle composition of claim 1, wherein
2	said conjugated lipid that inhibits aggregation of particles has the formula
	AY
2	I
	<del>-</del>

4	wherein:
5	A is a lipid moiety;
6	W is a hydrophilic polymer; and
7	Y is a polycationic moiety.
1	13 (original): The nucleic acid-lipid particle composition of claim 12, wherein W
2	is a polymer selected from the group consisting of PEG, polyamide, polylactic acid, polyglycolic
3	acid, polylactic acid/polyglycolic acid copolymers and combinations thereof, said polymer
4	having a molecular weight of about 250 to about 7000 daltons.
1	14 (original): The nucleic acid-lipid particle composition of claim 12, wherein Y
2	has at least 4 positive charges at a selected pH.
1	15 (original): The nucleic acid-lipid particle composition of claim 12, wherein Y
2	is a member selected from the group consisting of lysine, arginine, asparagine, glutamine,
3	derivatives thereof and combinations thereof.
1	16 (original): The nucleic acid-lipid particle composition of claim 12, wherein A
2	is a member selected from the group consisting of a diacylglycerolyl moiety, a dialkylglycerolyl
3	moiety, a N-N-dialkylamino moiety, a 1,2-diacyloxy-3-aminopropane moiety and a 1,2-dialkyl-
4	3-aminopropane moiety.
1	17 (original): The nucleic acid-lipid particle composition of claim 12, wherein W
2	is PEG.
1	18 (original): The nucleic acid-lipid particle composition of claim 12, wherein W
2	is a polyamide polymer.
1	19 (original): The nucleic acid-lipid particle composition of claim 12, wherein W
)	has a molecular weight of about 250 to about 2000 daltons.

1 20 (original): The nucleic acid-lipid particle composition of claim 17, having the 2 general structure of Formula II:

$$A - \left(X - (CH_2 - CH_2 - O)_n - Z\right) - Y$$

II

4 wherein

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X is a member selected from the group consisting of a single bond or a functional group covalently attaching said lipid to at least one ethylene oxide unit;

Z is a member selected from the group consisting of a single bond or a functional group covalently attaching said at least one ethylene oxide unit to a cationic group; and n is an integer having a value of between about 6 to about 50.

1 21 (original): The nucleic acid-lipid particle composition of claim 20, wherein

X is a member selected from the group consisting of a single bond,

3 phosphatidylethanolamino, phosphatidylethanolamido, phosphoro, phospho,

4 phosphoethanolamino, phosphoethanolamido, carbonyl, carbamate, carboxyl, carbonate, amido,

thioamido, oxygen, sulfur and NR, wherein R is a hydrogen or alkyl group.

1 22 (original): The nucleic acid-lipid particle composition of claim 20, wherein

Z is a member selected from the group consisting of a single bond,

3 phosphatidylethanolamino, phosphatidylethanolamido, phosphoro, phospho,

4 phosphoethanolamino, phosphoethanolamido, carbonyl, carbamate, carboxyl, carbonate, amido,

thioamido, oxygen, sulfur and NR, wherein R is a hydrogen or alkyl group.

1 23 (original): The nucleic acid-lipid particle composition of claim 20, wherein

2 A is a diacylglycerolyl moiety;

3 X is phosphoethanolamido;

Z is NR, wherein R is a hydrogen atom; and

5	Y is a member selected from the group consisting of about 1 to about 10 basic
5	amino acids or derivatives thereof.
1	24 (original): The nucleic acid-lipid particle composition of claim 23, wherein
2	A is a diacylgercerolyl moiety having 2 fatty acyl chains, wherein each acyl chain
3	is independently between 2 and 30 carbons in length and is either saturated or has varying
4	degrees of saturation.
l	25 (original): The nucleic acid-lipid particle composition of claim 23, wherein
2	Y is a member selected from the group consisting of lysine, arginine, asparagine,
3	glutamine, derivatives thereof and combinations thereof.
l	26 (original): The nucleic acid-lipid particle composition of claim 23, wherein
2	A is a diacylgercerolyl moiety having 2 fatty acyl chains, wherein each acyl chain
3	is a saturated C-18 carbon chain; and
1	Y is a cationic group having 4 lysine residues or derivatives thereof.
l	27 (original): The nucleic acid-lipid particle composition of claim 1, wherein
2	said conjugated lipid that inhibits aggregation of particles is a PEG-lipid
l	28 (original): The nucleic acid-lipid particle composition of claim 27, wherein
2	said PEG-lipid is PEG-ceramide.
l	29 (original): The nucleic acid-lipid particle composition of claim 28, wherein
2	the ceramide of said PEG-ceramide comprises a fatty acid group having about 8 to about 20
3	carbon atoms.
l	30 (original): The nucleic acid-lipid particle composition of claim 28, wherein
2	said PEG-lipid is PEG-phosphatidylethanolamine.

1	31 (original): The nucleic acid-lipid particle composition of claim 1, wherein
2	said conjugated lipid that inhibits aggregation of particles is an ATTA-lipid.
1	32 (original): The nucleic acid-lipid particle composition of claim 1, wherein
2	said nucleic acid is selected from the group consisting of a plasmid, an antisense oligonucleotide
3	and a ribozyme.
1	33 (currently amended): A method of introducing a nucleic acid into a cell, said
2	method comprising:
3	contacting said cell with a nucleic acid-lipid particle composition, said particle
4	composition comprising:
5	(a) a nucleic acid-lipid particle comprising a cationic lipid, a conjugated lipid that
6	inhibits aggregation of particles, and a nucleic acid, wherein said nucleic acid is encapsulated in
7	the a lipid bilayer of said nucleic acid-lipid particle, and wherein said conjugated lipid that
8	inhibits aggregation of particles is a member selected from the group consisting of a PEG-lipid,
9	an ATTA-lipid and a cationic-polymer-lipid conjugate having the formula
	AY
10	I
11	wherein:
12	A is a lipid moiety;
13	W is a hydrophilic polymer; and
14	Y is a polycationic moiety; and
15	(b) an endosomal membrane destabilizer, wherein said endosomal membrane
16	destabilizer is Ca <sup>++</sup> ion.
1	34 (original): The method of introducing a nucleic acid into a cell of claim 33,
2	wherein said endosomal membrane destabilizer is outside said nucleic acid-lipid particle.

35 (original): The method of introducing a nucleic acid into a cell of claim 33, 1 wherein said endosomal membrane destabilizer is Ca<sup>++</sup> ion. 2 1 36 (original): The method of introducing a nucleic acid into a cell of claim 35, wherein the concentration of Ca<sup>++</sup> ion is from about 0.1 mM to about 100 mM. 2 1 37 (original): The method of introducing a nucleic acid into a cell of claim 36, wherein the concentration of Ca<sup>++</sup> ion is from about 1 mM to about 20 mM. 2 1 38 (original): The method of introducing a nucleic acid into a cell of claim 33, 2 wherein said particle has a median diameter of less than about 150 nm. 1 39 (original): The method of introducing a nucleic acid into a cell of claim 33, 2 wherein said cationic lipid is a member selected from the group consisting of N,N-dioleyl-N,N-3 dimethylammonium chloride (DODAC), N,N-distearyl-N,N-dimethylammonium bromide (DDAB), N-(1-(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTAP), N-(1-4 (2,3-dioleyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTMA), and N,N-dimethyl-2,3-5 6 dioleyloxy)propylamine (DODMA), and combinations thereof. 1 40 (original): The method of introducing a nucleic acid into a cell of claim 33, 2 wherein said particle further comprises an additional noncationic lipid. 1 41 (original): The method of introducing a nucleic acid into a cell of claim 40, 2 wherein said noncationic lipid is selected from the group consisting of DOPE, POPC, and EPC. 1 42 (original): The method of introducing a nucleic acid into a cell of claim 33, wherein said particle comprises a functional group that facilitates Ca<sup>++</sup> ion chelation. 2 1 43 (original): The method of introducing a nucleic acid into a cell of claim 33, 2 wherein said conjugated lipid that inhibits aggregation of particles has the formula

## A----Y

3	1
4	wherein:
5	A is a lipid moiety;
6	W is a hydrophilic polymer; and
7	Y is a polycationic moiety.
1	44 (original): The method of introducing a nucleic acid into a cell of claim 43,
2	wherein W is a polymer selected from the group consisting of PEG, polyamide, polylactic acid
3	polyglycolic acid, polylactic acid/polyglycolic acid copolymers and combinations thereof, said
4	polymer having a molecular weight of about 250 to about 7000 daltons.
1	45 (original): The method of introducing a nucleic acid into a cell of claim 43,
2	wherein Y has at least 4 positive charges at a selected pH.
1	46 (original): The method of introducing a nucleic acid into a cell of claim 43,
2	wherein Y is a member selected from the group consisting of lysine, arginine, asparagine,
3	glutamine, derivatives thereof and combinations thereof.
1	47 (original): The method of introducing a nucleic acid into a cell of claim 43,
2	wherein A is a member selected from the group consisting of a diacylglycerolyl moiety, a
3	dialkylglycerolyl moiety, a N-N-dialkylamino moiety, a 1,2-diacyloxy-3-aminopropane moiety
4	and a 1,2-dialkyl-3-aminopropane moiety.
1	48 (original): The method of introducing a nucleic acid into a cell of claim 43,
2	wherein W is PEG.
1	49 (original): The method of introducing a nucleic acid into a cell of claim 43,
2	wherein W is a polyamide polymer.

II

Appl. No. 09/839,707 Amdt. dated October 26, 2004 Amendment under 37 CFR 1.116 Expedited Procedure Examining Group

- 50 (original): The method of introducing a nucleic acid into a cell of claim 43, wherein W has a molecular weight of about 250 to about 2000 daltons.
- 51 (original): The method of introducing a nucleic acid into a cell of claim 48, having the general structure of Formula II:

$$A - \left(X - (CH_2 - CH_2 - O)_n - Z\right) - Y$$

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4 wherein

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X is a member selected from the group consisting of a single bond or a functional group covalently attaching said lipid to at least one ethylene oxide unit;

Z is a member selected from the group consisting of a single bond or a functional group covalently attaching said at least one ethylene oxide unit to a cationic group; and

- n is an integer having a value of between about 6 to about 50.
- 52 (original): The method of introducing a nucleic acid into a cell of claim 51, wherein
- 3 X is a member selected from the group consisting of a single bond,
- 4 phosphatidylethanolamino, phosphatidylethanolamido, phosphoro, phospho,
- 5 phosphoethanolamino, phosphoethanolamido, carbonyl, carbamate, carboxyl, carbonate, amido,
- 6 thioamido, oxygen, sulfur and NR, wherein R is a hydrogen or alkyl group.
- 1 53 (original): The method of introducing a nucleic acid into a cell of claim 51,
- 2 wherein
- Z is a member selected from the group consisting of a single bond,
- 4 phosphatidylethanolamino, phosphatidylethanolamido, phosphoro, phospho,

phosphoethanolamino, phosphoethanolamido, carbonyl, carbamate, carboxyl, carbonate, amido, 5 thioamido, oxygen, sulfur and NR, wherein R is a hydrogen or alkyl group. 6 54 (original): The method of introducing a nucleic acid into a cell of claim 51, 1 2 wherein 3 A is a diacylglycerolyl moiety; 4 X is phosphoethanolamido; 5 Z is NR, wherein R is a hydrogen atom; and 6 Y is a member selected from the group consisting of about 1 to about 10 basic 7 amino acids or derivatives thereof. 1 55 (original): The method of introducing a nucleic acid into a cell of claim 54, 2 wherein 3 A is a diacylgercerolyl moiety having 2 fatty acyl chains, wherein each acyl chain 4 is independently between 2 and 30 carbons in length and is either saturated or has varying 5 degrees of saturation. 56 (original): The method of introducing a nucleic acid into a cell of claim 54, 1 2 wherein Y is a member selected from the group consisting of lysine, arginine, asparagine, 3 4 glutamine, derivatives thereof and combinations thereof. 1 57 (original): The method of introducing a nucleic acid into a cell of claim 54, 2 wherein 3 A is a diacylgercerolyl moiety having 2 fatty acyl chains, wherein each acyl chain 4 is a saturated C-18 carbon chain; and 5 Y is a cationic group having 4 lysine residues or derivatives thereof. 58 (original): The method of introducing a nucleic acid into a cell of claim 33, 1 2 wherein said conjugated lipid that inhibits aggregation of particles is a PEG-lipid.

68 (cancelled)

59 (original): The method of introducing a nucleic acid into a cell of claim 58, 1 2 wherein said PEG-lipid is PEG-ceramide. 60 (original): The method of introducing a nucleic acid into a cell of claim 59, 1 2 wherein the ceramide of said PEG-ceramide comprises a fatty acid group having about 8 to about 3 20 carbon atoms. 61 (original): The method of introducing a nucleic acid into a cell of claim 59, 1 wherein said PEG-lipid is PEG-phosphatidylethanolamine. 2 1 62 (original): The method of introducing a nucleic acid into a cell of claim 33, 2 wherein said conjugated lipid that inhibits aggregation of particles is an ATTA-lipid. 63 (original): The method of introducing a nucleic acid into a cell of claim 33, 1 2 wherein said nucleic acid is selected from the group consisting of a plasmid, an antisense 3 oligonucleotide, and a ribozyme. 64 (original): A method for inducing H<sub>II</sub> phase structure in a lipid bilayer, said 1 method comprising: contacting said lipid bilayer with an endosomal membrane destabilizer, 2 3 thereby inducing H<sub>II</sub> phase structure in a lipid bilayer. 65 (original): The method for inducing H<sub>II</sub> phase structure of claim 64, wherein 1 2 said lipid bilayer comprises DOPC:DOPE:DOPS:Chol. 1 66 (original): The method for inducing H<sub>II</sub> phase structure of claim 64, wherein said endosomal membrane destabilizer is Ca<sup>++</sup> ion. 2 67 (original): The method for inducing H<sub>II</sub> phase structure of claim 66, wherein 1 Ca<sup>++</sup> ion acts in concert with low levels of the cationic lipid to trigger H<sub>II</sub> phase formation. 2